REMARKS

1. The Amendments, the Support Therefor, and Basis for Entry

Four claims (6, 19, 32, and 45) have been canceled, no new claims have been added, and claims 1, 7, 9, 11, 14, 20, 22, 24, 27, 33, 35, 37, 40, 46, 48, and 50 have been amended to leave claims 1-5, 7-18, 20-31, 33-44 and 46-52 in the application. No new matter has been added by the amendments or new claims. In claims 1, 14, 27, 40, support for "at least 40 base pairs" can be found at page 7 line 21; "intercalating dye", page 8 line 25; and "temperature", page 8 line 27 onward. In claims 9, 22, 35, 48, support for "salt concentration less than 200 mM" can be found at page 13 lines 1-6.

As per 37 CFR §1.116(b) and MPEP 714.12/714.13, the amendments place the case either in condition for allowance or in better form for appeal, and thus are believed to be suitable for entry. As will be discussed below (and as supported by the accompanying Declaration), it is believed that the scope of the claims versus the prior art has been misapprehended, and that the claims are allowable (particularly in view of the further amendments to differentiate them from the prior art).

2. Sections 1-2 of the Office Action: Rejection of Claims 1-6, 8, 10-19, 21, 23-32, 34, 36-45, 47, and 49-52 under 35 USC §103(a) in view of *Drobyshev et al* (Gene (1997) 188:45-52) and U.S. Patent 6,174,670 to *Wittwer*

The present invention is concerned with polymorphism scoring methods that are simple and robust enough for automated high volume screening. Previously reported methods of allele discrimination on a solid surface suffered from low signal strength, high background and poor discrimination between alleles. Low signal strength meant that long exposure times were necessary in order to detect signal bound to the surface. These long exposure times dictated that the solid phase hybridization methods could only be used in a static fashion to determine hybridization at a particular temperature. Dynamic, real-time measurement of hybridization was not possible.

Both *Drobyshev et al* and *Wittwer* can be seen as alternative approaches to these previous methods, and both are aimed at achieving greater sensitivity than could be achieved with solid surface hybridization methods for allele discrimination. *Drobyshev et al* teaches the hybridization of fragmented RNA molecules to oligonucleotides immobilized within polyacrylamide gel pads. The RNA molecules are fluorescently labeled and the amount of label bound to the immobilized oligonucleotide is measured using fluorescence microscopy to produce a melting curve. To provide a standard reference Td, an oligonucleotide may be used instead of an RNA molecule. The increased binding capacity and hybridization characteristics of oligonucleotides immobilized in three dimensions within the gel lead to dramatic improvements in sensitivity.

Wittwer teaches real-time monitoring of hybridization between nucleic acid strands in a PCR reaction using a variety of formats including double stranded DNA dyes, pairs of FRET labeled oligonucleotide probes and single FRET labeled probes with FRET labeled target sequence. As amplification by PCR produces large amounts of target nucleic acid, all of which is assayed, the requirement for sensitivity in monitoring the hybridization of this target is reduced.

However, neither *Wittwer* or *Drobyshev et al* describes ***solid-phase*** nucleic acid hybridization, which is distinct from the approaches described in either of the above. The claimed feature of "a single DNA strand of a double stranded DNA of at least 40 base pairs containing the locus of a variation bound to a solid surface" is not taught in either *Drobyshev et al* or *Wittwer*. Furthermore, this feature would not be obvious to an ordinary artisan from the combined teachings of these documents. *Wittwer* is entirely concerned with PCR reactions in solution and there is no teaching of the immobilization of any of the components of these reactions. *Drobyshev et al* employs an array of 10mer oligonucleotides which are immobilized within a polyacrylamide gel. While the Examiner alleges that an ordinary artisan would consider a polyacrylamide gel to be a "solid surface", *a polyacrylamide gel is not a "solid surface" as the term is understood in the art, and the presence of this* ***specifically recited*** *feature provides an important distinction over the prior art*. Consider that where a claim term is an everyday word which is not assigned a special or limited meaning by the specification, a dictionary should be used to determine the meaning that should be given to the term. *Optical Disc Corp. v. Del Mar Avionics*, 54 USPQ2d 1289, 1295

(Fed. Cir. 2000); *Vanguard Products Corp. v. Parker Hannifin Corp.*, 57 USPQ2d 1087, 1089 (Fed. Cir. 2000). Here, a "surface" is defined as:

- the outer or top part or layer of something
  (Cambridge International Dictionary of English, http://dictionary.cambridge.org/define.asp?key=surface*1+0)

- the exterior or upper boundary of an object or body
  (Merriam Webster's Collegiate Dictionary, 10th Edition, http://www.m-w.com/cgi-bin/dictionary?book=Dictionary&va=surface)

- 1a. The outer or the topmost boundary of an object. b. A material layer constituting such a boundary.
  (The American Heritage Dictionary of the English Language, http://www.bartleby.com/61/29/S0912900.html)

- the outer part or external aspect of an object
  (Dorland's Illustrated Medical Dictionary, http://www.mercksource.com)

- The exterior part of anything that has length and breadth; one of the limits that bound a solid, especially. The upper face; superficies; the outside.
  (The On-line Medical Dictionary, http://cancerweb.ncl.ac.uk/cgi-bin/omd?surface)

Note that *Drobyshev et al* clearly states on page 48 that the "[p]olyacrylamide gel provides a stable three dimensional support for immobilized oligonucleotides" (first full paragraph). The immobilized oligonucleotides are thus arranged *within* the body of the gel pad *in a three dimensional arrangement*. Oligonucleotides immobilized in this way are not bound to a "surface" as that term is commonly understood to an ordinary artisan. Additionally, the three dimensional arrangement of oligonucleotides is essential to the working of the *Drobyshev et al* method and is not simply an equivalent to a single layer of oligonucleotides bound to a solid surface. For example, page 48 of *Drobyshev et al* states (at first column, midway down):

> The polyacrylamide gel provides more than 100 times greater capacity for three dimensional immobilization of oligonucleotides than does a two dimension glass surface. The high concentration of immobilized oligonucleotides facilitates the discrimination of mismatch duplexes and enhances the sensitivity of measurements on the microchips.

The polyacrylamide gel is thus used in *Drobyshev et al precisely because it is not a solid surface*, in order to immobilize oligonucleotides in a three dimensional array to increase binding capacity and thus sensitivity. This further demonstrates that an ordinary artisan would not modify *Drobyshev et al* to meet the claimed invention; see MPEP 2143.01 (subsection entitled "The Proposed Modification Cannot Render The Prior Art Unsatisfactory For Its Intended Purpose").

The Examiner has cited US02/0109841 as evidence that the term "solid surface" may encompass a polyacrylamide gel. However, US02/0109841 relates to a scanning spectrophotometer for use in detecting fluorescence in liquid samples. This concerns the field of spectrophotometry, which is an entirely different field to that of the present invention. It is noted that the US search classification (356/318) of US02/0109841 is completely different from that of the other cited art (classification 435/6). Additionally, note that column 4 of US02/0109841 refers to "collecting the emission light from the sample in a microtiter well or on a two dimensional surface such as a glass microscope slide, polyacrylamide gel, silicon microarray or other solid surfaces". In the context of collecting emission light, slides, gels or microarrays are conveniently approximated to a two dimensional surface, as the flat shape of these bodies means that emission will be largely through the upper face relative to the narrow edges. However, whilst this approximation to a two dimensional surface is adequate in a macroscopic context, it is not useful in considering molecular events in the field of molecular biology, where a "narrow edge" may represent a significant dimension.

In the molecular hybridizations described in *Drobyshev et al*, the gel pads provide a three dimensional support for the immobilized oligonucleotides: it is stated several times and is, in fact, crucial to the method, as it provides an improvement in sensitivity of two orders of magnitude. A body that contains within it a three dimensional array of immobilized oligonucleotides is not a "surface" and would not be so considered by an ordinary artisan. Oligonucleotides arranged within the body of a gel pad are not "bound to a surface" in the same way that a submarine

submerged under the sea is not "at the surface", whereas a boat floating on the sea is "at the surface". Further evidence in support of this position is provided in the enclosed Kwok declaration.

Given that a gel represents a phase intermediate between solid and liquid, and is therefore neither solid nor liquid, an ordinary artisan would not consider the gel pads of *Drobyshev et al* to fulfill either of the requirements of the present claims (i.e. the gels are not solid and the oligonucleotides are not bound to a surface of the gel). The combined teaching of *Drobyshev et al* and *Wittwer* is therefore deficient in failing to teach "a single DNA strand bound to a solid surface". Any combination of these teachings by a person of ordinary skill would, in fact, result in nucleic acid molecules were either immobilized within a gel or free in solution. Neither of these possibilities is encompassed by the present claims.

A person of ordinary skill would find nothing in the combined teaching of *Drobyshev et al* and *Wittwer* which would offer a solution to the fundamental problem of low sensitivity in monitoring hybridization on a solid surface. Hybridization on a solid surface has entirely different physico-chemical properties from hybridization in solution or within the body of a gel. Nucleic acid molecules bound to a solid surface are tightly packed in the same orientation, and each molecule interacts with its neighbors. These interactions may affect the hybridization, for example causing high background: signal ratios. In a liquid, however, the nucleic acid molecules are not aligned and are well spaced, minimizing inter-molecular interactions. Hybridization in a gel phase has properties very similar to that of a liquid phase. *Drobyshev et al* itself specifically states that "hybridization within the gel looks more like a liquid phase than a solid phase reaction" (page 48, 4th sentence of section 2.3). Neither *Drobyshev et al* nor *Wittwer* therefore addresses the problems intrinsic in performing and monitoring hybridization on a solid surface, and the skilled person's expectation that solid phase hybridization cannot be monitored in a sufficiently sensitive manner for dynamic (i.e. real-time) reading is unaltered by the combined teaching of these disclosures.

Apart from the foregoing, the recited intercalating agent is a further basis for the unobviousness of the claimed invention. The Examiner alleges that the substitution of the

fluorescent labels used in *Drobyshev et al* for the SYBR Green I marker used in examples in *Wittwer* would represent an obvious modification of the *Drobyshev et al* approach. However, *Drobyshev et al* does not mention or suggest the possibility of replacing the fluorescent label with any intercalating agent for the determination of the Tm of the probe/target complex. An artisan would, in fact, foresee various problems in using such intercalating agents in such a method. The double stranded sequence in *Drobyshev et al* (10bp: 1 turn of DNA helix) is considerably shorter than in *Wittwer* (110bp Example 2) and it would not be clear to an artisan whether an intercalating agent, in particular a double strand specific intercalating agent, would bind to such a short sequence or whether the sensitivity provided, in the event of successful binding, would be sufficient for detection over the background signals from inter-target interactions. For allele discrimination, sudden and consistent denaturation is required to produce detectable differences in melting curves between wild-type and mismatch probe/target complexes. The effect of an intercalating agent as opposed to a labeled probe, on the denaturation characteristics of the probe/target complex, would not be predictable to an artisan prior to the present invention. Replacing labeled target nucleic acid molecules with an intercalating agent would therefore involve considerable additional experimentation with no guarantee that the intercalating agent would work effectively in this context. Given that the signal might be enhanced in accordance with the teachings of *Drobyshev et al* by incorporating dUTP-fluorescein into the RNA, instead of simply adding a single 3' fluorescein label, an artisan would not be motivated to attempt to employ intercalating agents.

Furthermore, motivation to attempt to employ an intercalating agent would not be obtained from the teachings of *Wittwer*. In *Wittwer*, the melting curves using the double strand specific dye are used to discriminate between different PCR products (i.e. double stranded DNA molecules of completely different sequences) in Examples 14 to 20. However, *Wittwer* goes on to state: "when sequence specific detection and quantification are desired, resonance energy probes can be used instead of double-strand-specific DNA dyes" (column 42 lines 52-55). Accordingly, Examples 21 to 24, which show single base mismatch detection, employ two DNA bound FRET fluorophores not double-strand-specific DNA dyes. A person of ordinary skill is thus taught by

Wittwer that such dyes are not suitable for allelic discrimination and that a pair of DNA-bound FRET fluorophores should be used when single base mismatch detection is required. Were a person of ordinary skill to attempt to modify the method of *Drobyshev et al* in accordance with the teaching of *Wittwer*, he would therefore seek to employ pairs of DNA-bound FRET fluorophores, and would not employ a double-strand-specific DNA dye. A method so modified would not fall within the present claims.

The Examiner further alleges that *Wittwer* teaches a method which comprises monitoring the formation or dissociation of a complex consisting of (a), (b) and (c). This is not the case. Complexes which form as part of PCR necessarily comprise the additional component of a thermostable DNA polymerase enzyme, which tracks along the template strand synthesizing the second strand. Notwithstanding the presence or absence of thermostable DNA polymerase, the PCR products analyzed in the melting curve analysis shown for example in Figure 37 of *Wittwer* are double stranded DNA molecules and components (a) and (b) of the complex of the present claims are not present. There is therefore no teaching in either *Drobyshev et al* or *Wittwer* of a complex as set out in the present claims.

The presently claimed method relates to the hybridization of an allele specific oligonucleotide probe in solution with a single DNA strand of a double stranded DNA (i.e. a target molecule) bound to a solid surface. This configuration is not found in *Drobyshev et al*, where the oligonucleotide probe is immobilized and the target RNA is free in solution, or in *Wittwer*, which fails to teach immobilization of either probes or target. The presently claimed arrangement of probe and target would not be obvious to an artisan from the teachings of *Drobyshev et al* and *Wittwer*, taken either individually or together.

For the reasons explained above, the combination of *Drobyshev et al* and *Wittwer* fails to teach a complex consisting of features (a), (b) and (c) of the present claims, nor does it provide any expectation that hybridization characteristics of this complex, as monitored by a double-strand specific dye, might be suitable (in terms of signal strength and signal/background ratio) for routinely discriminating with high accuracy between different alleles of a nucleic acid sequence

in a dynamic (real-time) assay format. The present claims are therefore unobvious over *Drobyshev et al* in the light of *Wittwer*, and withdrawal of the §103(a) rejections is requested.

3. Section 3 of the Office Action: Rejection of Claims 1-8, 10-21, 23-34, 36-47, and 49-52 under 35 USC §103(a) in view of *Drobyshev et al* (Gene (1997) 188:45-52, U.S. Patent 6,174,670 to *Wittwer*, and U.S. Patent 6,048,690 to *Heller et al*

For the reasons described above, the combined teachings of *Drobyshev et al* and *Wittwer et al* are deficient in teaching the features of the presently claimed methods. *Heller et al* does not remedy these deficiencies and the combination of *Drobyshev et al*, *Wittwer* and *Heller et al* also fails to teach the features of the presently claimed methods. *Heller et al* describes the use of an electric field to induce perturbations in the fluorescence of a labeled probe hybridized to a oligonucleotide which is bound to a chip via a biotin/streptavidin interaction. However, the present methods employ heating to denature nucleic acid complexes. Immobilization via a non-covalent protein/cofactor interaction such as biotin/streptavidin would be considered to be unsuitable by an artisan for use at high temperature because of the possibility of streptavidin denaturation. The recognition by the present inventors that the biotin/streptavidin interaction was in fact suitable for attachment of nucleic acids at high temperatures and would provide sufficient binding capacity for dynamic reading was unexpected at the time of the invention. Since the combination of *Drobyshev et al* in view of *Wittwer et al*, and further in view of *Heller et al*, fails to suggest the features of the claimed methods, withdrawal of the rejections is requested.

4. Section 4 of the Office Action: Rejection of Claims 1-6, 8-19, 21-32, 34-45, and 47-52 under 35 USC §103(a) in view of *Drobyshev et al* (Gene (1997) 188:45-52), U.S. Patent 6,174,670 to *Wittwer*, and U.S. Patent 5,789,167 to *Konrad et al.*

For the reasons described above, the combined teachings of *Drobyshev et al* and *Wittwer* are deficient in teaching the features of the presently claimed methods. *Konrad et al* does not remedy these deficiencies, and the combination of these teachings with the teaching of *Konrad et al* also fails to teach the features of the presently claimed methods.

DNA buffer systems generally use high salt concentrations in order to stabilize DNA. This is evidenced on column 14 line 64 of *Konrad et al*, in which hybridization is performed in 300mM NaCl. The present inventors have recognized that in the presence of duplex specific dyes such as SYBR Green 1, high levels of salt lead to displacement of the dye and thus desensitization of the assay. For this reason, salt levels of around 50mM or less are preferred in the present methods, with a maximum upper limit of 200mM (first paragraph of page 13 of the present specification). A buffer which combines HEPES and low salt is not taught in either *Konrad, Drobyshev et al* or *Wittwer* and a person of ordinary skill would not expect such a buffer to be useful for nucleic acid hybridization. This feature of the present claims is therefore unobvious over the combined disclosures of these documents, and withdrawal of the rejection is requested.

5. In Closing

If any questions regarding the application arise, please contact the undersigned attorney. Telephone calls related to this application are welcomed and encouraged. The Commissioner is authorized to charge any fees or credit any overpayments relating to this application to deposit account number 18-2055.

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ATTACHMENTS:

- Amendment Sheet ("Marked-Up" Copy) Showing Changes to Application
- Kwok Declaration

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Serial No.:	09/755,747	Group Art Unit:	1637
Filing Date:	January 5, 2001	Examiner:	Fredman, J.
Applicants:	Brookes et al.	Atty. Docket:	40225.000
Title:	**DETECTION OF NUCLEIC ACID POLYMORPHISM**		

AMENDMENT SHEET ("MARKED-UP" COPY) SHOWING CHANGES TO APPLICATION (37 CFR §§1.121(b)(1)(iii); (c)(ii))

(To Accompany Response to September 11, 2002 Office Action)

In accordance with 37 CFR §§1.121(b)(iii) and (c)(ii), following are the amendments made to the specification and/or claims of the above-noted application.

- All deletions are indicated by brackets **[like so]** and all additions are indicated by underlining **like so**.
- The additions and deletions are made with respect to the application as it is understood to exist prior to entry of this amendment (i.e., any amendments are made with respect to the previous version).
- While 37 CFR §§1.121(b)(1)(iii) and (c)(1)(ii) does not require that new and canceled paragraphs and claims be supplied on this "marked-up" copy, such new additions and cancellations are nevertheless provided below to aid the reviewer's understanding.

IN THE CLAIMS:

Claims 6, 19, 32, and 45 are canceled without prejudice to further prosecution of these claims in one or more continuing applications.

Claims 1, 7, 9, 11, 14, 20, 22, 24, 27, 33, 35, 37, 40, 46, 48, and 50 are amended as follows:

1. **[TWICE AMENDED]** A method of detecting DNA variation by monitoring the formation or dissociation of a complex consisting of:
   (a) a single DNA strand of a double stranded DNA **of at least 40 base pairs** containing the locus of a variation bound to a solid surface,
   (b) an oligonucleotide or DNA analogue probe specific for one allele of the variation and capable of hybridizing to the single strand (a) to form a DNA duplex,
   (c) **an intercalating dye [a marker]** specific for the DNA duplex structure of (a) plus (b) which forms a complex with the said duplex and reacts uniquely when interacting within the DNA duplex,

   which method comprises:
   (1) continually measuring an output signal indicative of interaction of the **dye [marker]** with duplex formed from the strand (a) and probe (b), and
   (2) recording the **temperature [conditions]** at which a change in reaction output signal occurs which is attributable to formation or dissociation of the complex and is thereby correlated with the strength with which the probe (b) has hybridized to the single strand (a).

7. **[AMENDED]** A method according to claim **1 [6]**, in which **the single strand is bound to the solid surface [attachment is]** by a biotin/streptavidin type interaction.

9. **[AMENDED]** A method according to claim 8, in which the buffer solution is Hepes buffer **having a salt concentration less than 200 mM**.

11. **[AMENDED]** A method according to claim 1, in which **[the single strand is derived from a]** double stranded DNA **is a** product of PCR amplification of a target sequence.

14. **[TWICE AMENDED]** A method of detecting DNA variation which comprises bringing together:

(a) a single DNA strand of a double stranded DNA **of at least 40 base pairs** containing the locus of a variation bound to a solid surface,

(b) an oligonucleotide or DNA analogue probe specific for one allele of the variation and capable of hybridizing to the single strand (a) to form a DNA duplex,

(c) **an intercalating dye [a marker]** specific for the DNA duplex structure of (a) plus (b) which forms a complex with the said duplex and reacts uniquely when interacting within the duplex,

thereby forming a complex consisting of the components (a), (b) and (c), wherein the components (a), (b), and (c) are brought together under conditions in which either

(i) the component (a) hybridizes to component (b) and the complex is formed with component (c), or

(ii) the components (a) and (b) do not hybridize and the complex with component (c) is not formed,

(2) thereafter steadily and progressively adjusting the **temperature [conditions of the environment]**, respectively, either

(i) to denature the formed DNA duplex and cause dissociation of the complex, or

(ii) to cause formation of the DNA duplex and resulting complex,

(3) continually measuring an output signal indicative of the extent of hybridization of (a) and (b) and resulting complex formation with (c), and

(4) recording the temperature at **[conditions in]** which a change of output signal occurs which is indicative of, respectively,

(i) dissociation of the complex, or

(ii) formation of the complex.

20. **[AMENDED]** A method according to claim **14 [19]**, in which **the single strand is bound to the solid surface [attachment is]** by a biotin/streptavidin type interaction.

22. **[AMENDED]** A method according to claim 21, in which the buffer solution is Hepes buffer **having a salt concentration less than 200 mM**.

24. **[AMENDED]** A method according to claim 14, in which the **[single strand is derived from a]** double stranded DNA **is a** product of PCR amplification of a target sequence.

27. **[TWICE AMENDED]** A method of detecting DNA variation which comprises:
(1) forming a complex consisting of:
(a) a single DNA strand of a double stranded DNA **of at least 40 base pairs** containing the locus of a variation bound to a solid surface,
(b) an oligonucleotide or DNA analogue probe specific for one allele of the variation hybridized to the single strand (a) to form a duplex, and
(c) **an intercalating dye [a marker]** specific for the DNA duplex structure of (a) plus (b) and which reacts uniquely when interacting within the DNA duplex, and
(2) continually measuring an output signal of the extent of the resulting reaction of the marker and the duplex while steadily increasing the **temperature [denaturing environment containing the complex]**,
(3) recording the **[conditions] temperature** at which a change in reaction output signal occurs **[(herein termed the denaturing point)]** which is attributable to dissociation of the complex and is thereby correlated with the strength with which the probe (b) has hybridized to the single strand (a).

33. **[AMENDED]** A method according to claim **27 [32]**, in which **the single strand is bound to the solid surface [attachment is]** by a biotin/streptavidin type interaction.

35. **[AMENDED]** A method according to claim 34, in which the buffer solution is Hepes buffer **having a salt concentration less than 200 mM**.

37. **[AMENDED]** A method according to claim 27, in which the **[single strand is derived from a]** double stranded DNA **is a** product of PCR amplification of a target sequence.

40. **[TWICE AMENDED]** A method of detecting DNA variation which comprises:
(1) bringing together:
(a) a single DNA strand of a double stranded DNA **of at least 40 base pairs** containing the locus of a variation bound to a solid surface,
(b) an oligonucleotide or DNA analogue probe specific for one allele of the variation and capable of hybridizing to the single strand (a) to form a DNA duplex,
(c) **an intercalating dye [a marker]** specific for the DNA duplex structure of (a) plus (b) and which reacts uniquely when interacting within the duplex,
the components (a), (b) and (c) being brought together prior to formation of the defined complex and under conditions in which (a) and (b) do not hybridize;
(2) steadily adjusting the **temperature [conditions of their environment]** to cause formation of the duplex and resulting complex consisting of components (a), (b), and (c), and
(3) measuring an output signal indicative of the occurrence of hybridization of (a) and (b) (herein termed the annealing point).

46. **[AMENDED]** A method according to claim **40 [45]**, in which **the single strand is bound to the solid surface [attachment is]** by a biotin/streptavidin type interaction.

48. **[AMENDED]** A method according to claim 47, in which the buffer solution is Hepes buffer **having a salt concentration less than 200 mM**.

50. **[AMENDED]** A method according to claim 40, in which the **[single strand is derived from a]** double stranded DNA **is a** product of PCR amplification of a target sequence.